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Recovery of Campylobacter from Segments of the Reproductive Tract of Broiler Breeder Hens

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SUMMARY. Three groups of >60-wk-old broiler breeder hens were assessed for the presence of *Campylobacter* within segments of the reproductive tract. In the first group, after stunned, the hens were bled, scalded, and defeathered, the reproductive tracts were aseptically excised from 18 hens, six from each of three adjacent floor pens that were feces positive for *Campylobacter.* The reproductive tract segments (infundibulum, magnum–isthmus, shell gland, vagina, and cloaca) were pooled by pen. In the second group, 10 individual hens were sampled from the pens; the reproductive tract was divided into the following segments: magnum, isthmus, shell gland, vagina, and cloaca. For the third group, hens were obtained from two commercial farms that had been determined to be feces positive for *Campylobacter,* and the reproductive tract was divided into five segments, as described for the second group. Segments of the reproductive tract were placed into sterile plastic bags and suspended 1:3 (w/v) in Bolton enrichment broth, and serial dilutions were plated (0.1 ml) onto Campy-Cefex agar. The agar plates were incubated at 42 C for 24 hr in a microaerobic atmosphere. In group 1, the pooled reproductive tract segments for hens from pen A were *Campylobacter* positive for the shell gland, vagina, and cloaca; hens from pen B were positive for the cloaca only; and hens from pen C were positive for the magnum–isthmus and cloaca. In the second group, 9 of 10 cloaca samples were *Campylobacter* positive. Commercial hens in group 3 had campylobacter-positive cloaca samples (12/12), vagina (10/12), shell gland (7/12), isthmus (2/12), and magnum (4/12). *Campylobacter* colonization of the reproductive tract of the hen could enable vertical transmission of *Campylobacter* from the hen to the chick.

RESUMEN. Reaislamiento de Campylobacter a partir de segmentos del tracto reproductor de gallinas reproductoras de engorde.

Se evaluó la presencia de *Campylobacter* en segmentos del tracto reproductor en tres grupos de gallinas reproductoras de engorde de 60 semanas de edad. En el primer grupo de gallinas, luego de tomar muestras de sangre, escaldarlas y retirarles las plumas, se tomaron asépticamente los tracto reproductores de 18 gallinas, seis de cada uno de tres corrales en piso adyacentes en los cuales se detectó la presencia de *Campylobacter* a partir de las heces. Se agruparon los tractos reproductores (infundibulum, magnum, istmo, útero, vagina y cloaca) por corral. En el segundo grupo, los tractos reproductores de un total de 10 gallinas de los tres corrales se dividieron en los siguientes segmentos: magnum, istmo, útero, vagina y cloaca. En el tercer grupo, se obtuvieron gallinas de dos granjas comerciales en las cuales se aisló el *Campylobacter* a partir de las heces y sus tractos reproductores se dividieron al igual que en el segundo grupo. Se depositaron los segmentos del tracto reproductor en bolsas plásticas estériles, se resuspendieron en una dilución 1:3 peso/volumen de caldo de cultivo enriquecido Bolton y se sembraron diluciones consecutivas (0.1 ml) en medio agar Campy-Cefex. Las placas de agar fueron incubadas a 42 C por 24 horas en una atmósfera microaeróbica. En el grupo 1, se observaron tractos reproductores positivos a *Campylobacter* en el útero, vagina y cloaca de las gallinas del corral A, en la cloaca de las gallinas del corral B y en el magnum, istmo y cloaca de las gallinas del corral C. En el grupo 2, 9 de 10 muestras de cloaca fueron positivas a *Campylobacter.* En las gallinas comerciales del grupo 3, se observó la presencia de *Campylobacter* en muestras de cloaca (12/12), vagina (10/12), útero (7/12), istmo (2/12) y

magnum (4/12). La colonización del tracto reproductivo de las gallinas puede facilitar la transmisión vertical del *Campylobacter* a la progenie.

Key words: *Campylobacter,* breeder hen, reproductive tract, vertical transmission, colonization

Abbreviation: CFU = colony-forming units

Campylobacter is considered to be the most common causative bacterial agent of human sporadic diarrhea or food poisoning (1). Colonization in the intestinal tract of poultry with human-derived strains of *Campylobacter* has been demonstrated (10,13). The transmission of *Campylobacter* from the breeder farm through the hatchery, to the broiler flock, and finally to the processing plant has recently been demonstrated by identification and characterization of *Campylobacter* isolates of clonal origin from each of these sources (5). Vertical transmission of *Campylobacter* from the hen to the chick through the egg has not been directly demonstrated. *Campylobacter* was recovered from the surface of only 2 of 226 unwashed eggshells (<1%) for feces-positive caged laying hens, but *Campylobacter* could not be recovered from the egg contents (6). The author (6) concluded that egg transmission of *Campylobacter* to either humans or chicks was unlikely. *Campylobacter* (10% positive) has been recovered 2 days after inoculation from both the eggshell membranes and contents when contaminated feces were applied to cracked eggshells that were held at 25 or 42 C (3). Similarly, *Campylobacter* could be recovered from eggshell membranes, but not from the yolk or albumen, after immersion inoculation at 10^7/ml (12). However, after 24 hr of incubation at 37 C, *Campylobacter* could no longer be recovered. Although *Campylobacter* will grow in both liquid egg yolk and whole egg with a generation time of 50 min (4), recovery of *Campylobacter* from processed table eggs or egg products has been unsuccessful (8). In one study, *Campylobacter* was not recovered from hatcheries (chick yolk sacs, hatchers, or chick boxes) although both the breeder and broiler flocks were *Campylobacter* positive (9). However, a recent report detected *Campylobacter* spp. in hatchery samples by polymerase chain reaction methodology (7).

Individually caged Japanese quail hens orally challenged with *Campylobacter jejuni* ($\log_{10}$ 9.6) resulted in the recovery of *Campylobacter* from both eggs and the reproductive tract (11). All hens were cloaca positive for *Campylobacter,* but only 28% of the eggshells and 4% of egg contents were positive. These researchers were successful in isolation of *Campylobacter* from the magnum–isthmus and shell gland segments of the reproductive tract but not the infundibulum.

The present study was undertaken to determine if it was possible to recover *Campylobacter* from segments of the reproductive tract of broiler breeder hens that were naturally feces positive for *Campylobacter.*

MATERIALS AND METHODS

Groups 1 and 2 were sampled 1 wk apart in the month of September and utilized broiler breeder hens from the same University of Georgia research flock that had been incubated, hatched, and reared in isolation at the university. Thirty-five hens were housed in each mating pen (2.4 × 3.6 m) partitioned into ⅔ elevated slats and ⅓ litter. For group 1, 2 hr after the morning feeding, six broilers breeder hens (66 wk old) were selected from each of three adjacent floor pens (A, B, C). The pens had tested feces positive for *Campylobacter* the previous week. Each hen was selected for the ability to evert the vagina, assuring active egg production status. The six hens were placed into each coop, by pen, and transported to the pilot processing plant. The six hens from one pen were processed together, requiring three batches at 15-min intervals. For the second group, five hens at 67 wk of age from pens A and C were placed into each coop, by pen, and transported to the pilot processing plant. Hens from pen B were not sampled in the second group because no hen from this pen had positive tract segments the previous week, and tract segments for each individual hen were to be cultured separately.

Group 3 was studied in April. On 2 days 1 wk apart, six broiler breeder hens (61 and 62 wk of age) were obtained from commercial broiler breeder farms that were feces positive the week prior to reproductive tract sampling. Housing was conventional for Georgia, and hens were cooped at the farm, transported to the pilot processing plant, and held overnight.

To minimize aerosolized feather-fecal contamination, hens were processed through defeathering. Hens

Table 1. Recovery of *Campylobacter* after enrichment for 24 hr (+ or −) from segments of the reproductive tracts of broiler breeder hens (groups 1 and 2).

Group	Pen[A]	Infundibulum	Magnum–isthmus	Shell gland	Vagina	Cloaca
1[B]	A	−	−	+	+	+
	B	−	−	−	−	+
	C	−	+	−	−	+
2[C]	A					
	Hens 1, 2, 4, 5	NS[D]	−	−	−	+
	Hen 3	NS	−	−	−	−
	C					
	Hen 6	NS	−	+	+	+
	Hens 7, 8, 9, 10	NS	−	−	−	+

[A]Values for pens A, B, and C in group 1 are the results of pooled samples from six hens per pen.
[B]Age = 66 wk, n = 18 hens.
[C]Age = 67 wk, n = 10 hens.
[D]NS = not sampled.

were electrically stunned while suspended in shackles, bled for 90 sec, scalded at 56.7 C for 120 sec, and then defeathered in a single pass through a five-bank picker for 30 sec.

Excision of reproductive tract. Each carcass was placed on its back, the abdominal cavity was opened aseptically, and the digestive tract was lifted out of the abdominal cavity to the right of the hen. The skin surrounding the vent was then encircled, the colon was clamped and transected, and the reproductive tract was lifted out of the abdominal cavity to the left of the hen. For group 1, the tract was then aseptically sectioned into the infundibulum, magnum–isthmus, shell gland, vagina, and cloaca. The location of any forming eggs was noted for each hen, and the contents were removed. Procedures for groups 2 and 3 were the same as for group 1 except that the infundibulum was discarded (because no positive sample had previously been detected for this segment) and the isthmus and magnum were isolated as discrete segments.

Isolation of *Campylobacter*. From the first group, each of the reproductive tract segments was placed into a pooled sample bag for each pen (A, B, and C). For the second and third groups, tract segments were placed into individual bags by hen. Sample bags and contents were held on ice until the addition of 1:3 (w/v) Bolton enrichment broth. Samples were stomached for 1 min, and serial dilutions were plated (0.1 ml) onto Campy-Cefex agar in duplicate (14). For enrichment, the remaining sample solutions were incubated for 4 hr at 37 C and then at 42 C for an additional 20 hr. After a total of 24 hr, these enriched samples (0.1 ml) were plated onto Campy-Cefex agar in duplicate. All plates were incubated at 42 C for 36 hr in a microaerobic atmosphere (5% O_2, 10% CO_2, 85% N_2). A representative number of presumptive *Campylobacter* colonies were confirmed by phase-contrast microscopic examination (to visualize characteristic motility and morphology) as well as latex agglutination with a test kit specific for *Campylobacter jejuni, Campylobacter coli,* and *Campylobacter lari* (Integrated Diagnostics, Inc., Baltimore, MD). For group 3, direct plate counts were enumerated.

RESULTS

In group 1, after enrichment, the pooled reproductive tract segments for hens from pen A were *Campylobacter* positive for the shell gland, vagina, and cloaca; hens from pen B were positive for the cloaca only; and hens from pen C were positive for the magnum–isthmus and cloaca (Table 1). In the second group, 9 of 10 cloaca samples were *Campylobacter* positive (Table 1). Hen 6 was *Campylobacter* positive in both the shell gland and vagina. Hen 6 was the only hen lacking a forming egg within the reproductive tract at the time of sample collection.

In group 3, all cloaca samples from both commercial flocks were *Campylobacter* positive by direct plating, resulting in enumeration from 1 to 1202 colony-forming units (CFU)/ml of broth (Table 2). *Campylobacter* was detected from the reproductive tract segments as follows: the vagina (10/12, five required enrichment), the shell gland (7/12, four required enrichment), the isthmus (2/12), and the magnum (4/12, three required enrichment). *Campylobac-*

Table 2. Recovery of *Campylobacter* from direct plating (CFU $\log_{10}$/ml of suspension) or enrichment (+ or −) for 24 hr from segments of the reproductive tracts of broiler breeder hens from commercial flocks (groups 3, presented by order of incidence).

Farm	Hen	Magnum	Isthmus	Shell gland	Vagina	Cloaca
1[A]	2	+[C]	1.30	1.77	2.15	3.76
	3	−	−	+	1.00	2.23
	1	−	−	−	+	3.63
	5	−	−	−	+	2.60
	4	−	−	−	+	1.70
	6	−	−	−	+	1.00
2[B]	2	1.78	1.95	1.70	2.15	3.55
	3	+	−	1.95	1.48	4.08
	1	+	−	+	+	2.15
	4	−	−	+	1.60	2.34
	6	−	−	+	−	1.48
	5	−	−	−	−	1.62
No. positive		4/12	2/12	7/12	10/12	12/12

[A]Age = 62 wk.
[B]Age = 61 wk.
[C]After enrichment in Bolton broth.

ter was detected in 92% (11/12) of the reproductive tracts collected from these commercial hens (at 61 and 62 wk of age).

DISCUSSION

Campylobacter was recovered from the terminal segments of the reproductive tract (magnum–isthmus, shell gland, vagina, and cloaca) of the broiler breeder hen from both university and commercial flocks. Hens from commercial flocks appeared to have higher levels and prevalence of recovery of *Campylobacter* than did those from the university flock. Enrichment of the samples from the commercial farms improved the detection rate from 11 of 48 positive tract segments (by direct plating) to 23 of 48 positive tract segments (after enrichment). The range in the level of *Campylobacter* recovered for the tract segments from the commercial hens was <10 CFU/ml (enrichment positive) to $\log_{10}$ 2.15 CFU/ml of suspension. These recovery values for the reproductive tract segments overlap those for the cloaca samples but were consistently lower than cloaca levels for each individual hen.

The pathway for *Campylobacter* to ascend the reproductive tract of hens is not documented. Reverse peristalsis is common in the hen's reproductive tract and results in yolks to full-formed shell eggs within the abdominal cavity. Spermatozoa are transported from the primary sperm storage glands located at the vaginal–shell gland junction to the secondary sperm storage glands located at the junction of the magnum and infundibulum. Examination of histologic tissue sections of the primary sperm storage glands, which were selectively stained for *Campylobacter,* revealed *Campylobacter* adjacent to spermatozoa within the glands for tract samples collected the day after insemination with *Campylobacter*-spiked semen (unpubl. data). Transport of *Campylobacter* from the primary to the secondary sperm storage glands has not yet been documented, but transport along with spermatozoa would provide a mechanism for exposure throughout the reproductive tract of the hen. The possibility that the reproductive tracts become positive for *Campylobacter* while suspended upside down during processing is not likely for the following reasons. First the reproductive tract of the hen is not straight but is coiled and convoluted as is the digestive tract. Second, *Campylobacter* was not recovered from the reproductive tract segments from the six hens in group 1, pen B although the cloaca sample was positive (Table 1). Hens from group 1, pen C were positive for the cloaca segment, negative for the adjacent vaginal and shell gland segments, but positive for the combined magnum–isthmus segment. These results would argue against retrograde of *Campylobacter* within the reproductive tract during processing. Fur-

thermore, in group 3, farm 2 (Table 2), *Campylobacter* was recovered from commercial hens 1 and 3 from the shell gland and magnum segments but not the intervening isthmus segments. Similarly, hen 6 had a positive cloaca and shell gland but the intervening vagina segment was negative. Finally, hen 5 had a positive cloaca sample but the entire reproductive tract was negative.

In single study (11), *Campylobacter* was recovered from the reproductive tract of quail hens after an oral challenge with *C. jejuni* ($\log_{10}$ 9.6) where the hens were housed in cages. All of the hens were colon positive for *Campylobacter,* and the authors were able to isolate *Campylobacter* from the magnum–isthmus and shell gland segments of the reproductive tract from three of seven hens. One of their hens had small white foci in the liver, and in another hen, the C1, C2, and C3 follicles of the ovary were *Campylobacter* positive, suggesting systemic *Campylobacter* infection. The authors (11) suggested that *Campylobacter* may contaminate hatching eggs by either entering the vascular system and thereby invading the ovary and developing follicles or ascending the reproductive tract from the cloaca to colonize the oviduct. In recent study (2) *Campylobacter* was recovered from 12 of 150 caged laying hens sampled from three farms. From four of the 12 hens, *Campylobacter* was recovered from the reproductive tract (vagina, isthmus, magnum, or infundibulum). They genotyped *Campylobacter* by *fla* typing and reported that consistently the same *fla* type was identified within an individual hen's reproductive tract, but only for two of the four hens did the *fla* type match that recovered from the cloaca or cecum. The authors (2) concluded that by distinguishing 10 *fla* types of *Campylobacter,* the laying flocks were subjected to multiple exposures of *Campylobacter.* One common source of *Campylobacter* exposure to chicks may be in the hatchery, as suggested by the ubiquitous detection of *Campylobacter* spp. in hatchery debris by polymerase chain reaction methods (7), which indicated that at least characteristic *Campylobacter* DNA was commonly present.

In the present study, the consistent recovery of *Campylobacter* from the cloaca of all commercial broiler breeder hens (12/12) and the high incidence of recovery from segments of these hens' reproductive tracts (10/12) suggest that there may be a greater prevalence of *Campylobacter* in conventional commercial broiler breeder houses than that reported for cage layer facilities. However, simply placing hens in wire cages appears not to prevent *Campylobacter* colonization of the digestive tract of broiler breeder hens. Feces samples from caged broiler breeder hens at 64 wk of age (caged since 18 wk of age) had a relatively high prevalence of *Campylobacter* at 16 of 25 hens positive (unpubl. data). The presence of *Campylobacter* within the reproductive tract of the broiler breeder hen for feces-positive flocks could potentiate vertical transmission of *Campylobacter* from the hen through the egg to the broiler chick. Our future experiments will investigate potential exogenous sources that may serve to transmit *Campylobacter* to the reproductive tract of the hen.

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